An investigation into the metabolism and toxicity of buspirone and nefazodone

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Idiosyncratic drug-induced liver injury (DILI) is becoming an increasing problem in drug development (Lee 2003). Nefazodone is an anti-depressant that was withdrawn from the market in 2004, following cases of rare, but severe, liver injury (Aranda-Michel, Koehler et al. 1999). In contrast, the structurally related drug, buspirone, an anxiolytic agent, has no reported cases of DILI despite undergoing a similar metabolism to nefazodone (Kalgutkar, Vaz et al. 2005). Three mechanisms of nefazodone toxicity have previously been reported, reactive metabolite formation, mitochondrial toxicity and BSEP inhibition. We aim to further elucidate the mechanism by which nefazodone is toxic using buspirone as a control.

Rat liver microsomes were isolated from Wistar rats to determine the covalent binding of buspirone and nefazodone. Microsome incubations were carried out at 1 mg/ml in phosphate buffer using 0.1 μCi of [14C]-labelled compound. Hepatocytes were isolated from male Wistar rats in a two step in situ collagenase perfusion method. The hepatocytes were incubated for 6 hours with a range of 0-500 μM buspirone or nefazodone and toxicity was assessed using trypan blue and MTS. Glutathione levels were also investigated. Determination of metabolites in microsomes and hepatocytes was performed using LC-MS/MS.

Buspirone demonstrated NADPH-dependent binding to microsomes (1.16% bound/mg protein SD±0.29%). Addition of the trapping agent glutathione significantly (p=0.0058) reduced covalent binding of buspirone (0.64% bound/mg protein SD±0.17). Nefazodone also appears to demonstrate NADPH-dependent covalent binding in microsomes that is reduced by the addition of glutathione.

Toxicity and metabolism were assessed in rat hepatocytes. At 500μM buspirone viability as percent of control was 88.5% (SD±6.5%), whereas 500μM nefazodone resulted in a 100% mortality rate. Further investigation using inhibitors of cytochrome P450 will determine whether this toxicity can be attributed to reactive metabolite formation. Glutathione depletion was seen to be a result of cell death rather than a cause of cell death (n=3).

Buspirone was metabolised to several mono and di-hydroxylated metabolites in microsomes and 19 metabolites were identified in hepatocytes. The major routes of metabolism are hydroxylation, N-dealkylation and glucuronidation.

In conclusion it was found that nefazodone is more toxic than buspirone in freshly isolated rat hepatocytes, but did not deplete glutathione. Future work will address whether mitochondrial toxicity or BSEP inhibition play a part in nefazodone toxicity.